Effect of ketamine on the physiological responses to combined hypoglycemic and psychophysical stress

Brett Melanson , Francesco Leri [*]

Department of Psychology and Collaborative Neuroscience, Program University of Guelph, Guelph, ON, Canada

ABSTRACT

There is evidence that hypoglycemic stress can interact with other stressors, and that ketamine can mitigate the impact of these stressors on behavior and physiology. The current study in male Sprague-Dawley rats investigated whether pre-treatment with 0, 10, or 20 mg/kg ketamine could modulate the interaction between hypoglycemia induced by 0 or 300 mg/kg 2-deoxy-D-glucose (2-DG) and the psychophysical stress of forced swimming (FSS; 6 sessions, 10 min/session) on serum concentrations of corticosterone (CORT) and the pro-inflammatory cytokine, tumor necrosis factor (TNF)-α. It was found that 2-DG enhanced the CORT response to an initial session of FSS, and this effect dissipated after multiple sessions. More importantly, animals displayed significantly higher levels of CORT and lower levels of TNF-α in response to a drug-free test swim conducted 1 week after exposure to the combined stressors, and these responses were not observed in rats that were pre-treated with ketamine. Overall, these findings indicate that ketamine has the potential to reduce the negative impact of interacting stressors on the biological reactivity of the hypothalamic-pituitary-adrenal axis and the immune system.

Introduction

There is increasing interest in understanding the link between metabolic and mood disorders (Anderson et al., 2001; Lustman and Clouse, 2005). Hypoglycemia is a physiological state characterized by low glucose utilization and widespread energetic stress (Harrell et al., 2016). Interestingly, hypoglycemia negatively impacts mood (Horman et al., 2018) and hedonic tone (Gold et al., 1995) in several species, and can interact with other stressors to enhance their effects on behavior and physiology (Deak et al., 2005; Remus et al., 2015). For example, Melanson et al. (2021) recently found that hypoglycemia induced by the glycolytic inhibitor, 2-deoxy-D-glucose (2-DG), interacted with forced swimming stress (FSS)—a psychophysical stressor (Balkan et al., 2012; Imbe and Kimura, 2015)—to amplify depressive-like behavioral responses in rats.

There is evidence that interacting hypoglycemic and psychophysical stressors can impact the hypothalamic-pituitary-adrenal (HPA) axis as well as components of the immune system. The HPA axis is a neuroendocrine system that is activated in response to many types of stressors including hypoglycemia (Razavi Nematollahi et al., 2009) and forced swimming (Dal-Zotto et al., 2000). Upon activation, the adrenal glands

release the glucocorticoid corticosterone (cortisol in humans; CORT) into the blood (Herman et al., 2016), which primarily functions to allocate energy resources toward vital organs (i.e., brain) that are required to overcome environmental threats (Herman et al., 2016; Kuo et al., 2015; Myers et al., 2013). Pro-inflammatory cytokines are signaling proteins of the immune system that regulate inflammation and the immune response, and which also respond to a variety of stressors (Carroll et al., 2011; López-López et al., 2016; Timberlake et al., 2019). For example, blood levels of the pro-inflammatory cytokines interleukin (IL)-6 and tumor necrosis factor (TNF)-α increase following exposure to hypoglycemia (Dréau et al., 2000; Drummond et al., 2018) as well as forced swimming (Himmerich et al., 2013). Moreover, pro-inflammatory cytokines, such as TNF-α, are implicated in a variety of physiological processes, like glucose metabolism (De Alvaro et al., 2004; Lorenzo et al., 2008) and activation of the HPA axis (Dunn, 2006), which are impacted by hypoglycemic (Engelsen et al., 1986; Tasker et al., 1992) and psychophysical stressors (Borsoi et al., 2015; Dal-Zotto et al., 2000).

Given the high prevalence of metabolic and mood disorders (Nouwen et al., 2019) and their significant burden of disease in North America (World Health Organization, 2016, 2017), it is important to

investigate pharmacological tools that may have clinical utility. Therefore, the current study investigated whether the glutamate *N*-methyl-D-aspartate (NMDA) receptor antagonist, ketamine, can modulate the interaction between hypoglycemia induced by 2-DG (Melanson et al., 2021) and psychophysical stress induced by FSS (Balkan et al., 2012) on serum levels of CORT and TNF-α. Typically, ketamine is used to induce anesthesia in surgical settings (Gao et al., 2016), but recent clinical studies have also demonstrated its utility as a rapid-acting antidepressant drug (Niciu et al., 2018; Phillips et al., 2019). Importantly, pre-clinical studies have shown that pre-treatment with another NMDA receptor antagonist, MK-801, can block hypoglycemia-induced apoptosis of neurons (Tasker et al., 1992; Wieloch, 1985). As well, pre-treating rodents with ketamine induces prophylaxis against future stressors (Amat et al., 2016; Brachman et al., 2016); and, lowers blood (Gonçalves et al., 2021; Zhan et al., 2020) and central (Zhang et al., 2016) levels of pro-inflammatory cytokines (Mastrodonato et al., 2020; Walker et al., 2015). Finally, ketamine also reverses depressive-like behaviors induced by hypoglycemic and psychophysical stressors (Melanson et al., 2021). In the current study, it was found that interacting hypoglycemic and psychophysical stress amplified CORT and TNF-α responses well after exposure to the combined stressors, and that pre-treatment with ketamine attenuated these effects. These findings indicate that ketamine has the potential to reduce the negative impact of interacting stressors on the biological reactivity of the HPA axis and immune system.

Experimental procedures

Subjects

A total of 66 male Sprague-Dawley rats (Charles River, QC) weighing between 250 and 275 g upon arrival to the laboratory were individually housed in standard rat cages (polycarbonate; $50.5 \times 48.5 \times 20$ cm) with bedding and standard environmental enrichment. Upon arrival, rats were given 1 week to acclimate to the animal facility and were maintained on a 12-h reverse light/dark schedule (lights off 0700 h, lights on 1900 h). All behavioral testing was conducted during the dark period. Tap water and chow (Teckblad Global 18% Rodent diet, Envigo RMS Inc., Indianapolis, IN, USA) were available ad libitum throughout all experiments. This study was approved by the Animal Care Committee of the University of Guelph and were carried out in accordance with the recommendations of the Canadian Council on Animal Care.

Apparatus

Forced swimming stress

The forced swimming stress protocol used in the current study has been previously described by Melanson et al. (2021). Briefly, FSS was conducted in four transparent Plexiglass cylinders in a dimly lit room, and each cylinder was filled with tap water (23 ± 1 °C) to a depth of 40 cm.

Tissue collection and analysis

Blood collection

Blood samples (approximately 250 μl per sample) were collected via saphenous vein using Sarstedt Microvette CB300 Capillary tubes (Fischer Scientific; Cat No: NC9059691) and allowed to coagulate at room temperature for at least 90 min. Whole blood was then centrifuged at 2000*g* for 15 min at 4 °C to separate serum. Aliquots of serum were pipetted into 1.5 ml Eppendorf Microcentrifuge tubes (Fisher Scientific; Cat No: 05-402) and stored at -80 °C until analysis. Blood samples for each test were collected within 2 min to avoid any potential influence of the stress caused by the restraining method used to collect each sample (Lovelock and Deak, 2017).

Competitive enzyme-linked immunosorbent assay

Serum concentrations of CORT were determined using competitive enzyme-linked immunosorbent assay (ELISA) kits purchased from R&D systems (Cat. No. KGE009; Minneapolis, MN). The competitive binding immunoassay was carried out according to the manufacturer's instructions to detect unknown concentrations of CORT in serum samples. Following a series of wash, incubation, and reaction development steps, the microplate was read at 450 nm to determine the optical density (O.D.) of each well. O.D. values were quantified relative to a standard curve that was run with each plate and concentrations are reported in ng/ml. Serum samples were run in duplicates with an intra-assay coefficient of variation (CV) of $< 10\%$ and an inter-assay CV of $< 15\%$.

Sandwich enzyme-linked immunosorbent assay

Serum concentrations of TNF-α were determined using sandwich ELISA kits purchased from R&D Systems (RTA00; Minneapolis, MN). The sandwich immunoassay was carried out according to the manufacturer's instructions to detect unknown concentrations of TNF-α in each sample. Following a series of wash, incubation, and reaction development steps, the microplate was read at 450 nm to determine the O.D. of each well. O.D. values were quantified relative to a standard curve run with each plate and concentrations are reported in pg/ml. Serum samples were run in duplicates with an intra-assay CV of $< 10\%$ and an inter-assay CV of $< 15\%$.

Drugs

2-DG (Sigma-Aldrich, St. Louis, MO) was dissolved in 0.9% saline (wt/vol) and injected subcutaneously (SC) at a volume of 1 ml/kg. The 300 mg/kg dose of 2-DG was chosen based on its aversive and stress-inducing characteristics reported by Horman et al. (2018). Moreover, a previous experiment in our laboratory that investigated the interaction between 0, 200, or 300 mg/kg 2-DG and FSS on CORT levels found that the most robust effect was at 300 mg/kg 2-DG (unpublished observations).

Ketamine hydrochloride (Narketan; 100 mg/ml) was diluted with 0.9% saline (wt/vol) and injected intraperitoneally (10 or 20 mg/kg, IP) at a volume of 1 ml/kg. Doses for ketamine were chosen based on their ability to reverse stress-induced immobility in the rat forced swim test (Carrier and Kabbaj, 2013; Zhang et al., 2016) and their prophylactic effects against stress in rodents (Amat et al., 2016; Melanson et al., 2021).

Procedures

Rats in the Swim stress condition were randomly assigned to one of six experimental groups ($n = 8$ per group): (1) 0 mg/kg 2-DG and 0 mg/kg Ketamine; (2) 300 mg/kg 2-DG and 0 mg/kg Ketamine; (3) 0 mg/kg 2-DG and 10 mg/kg Ketamine; (4) 300 mg/kg 2-DG and 10 mg/kg Ketamine; (5) 0 mg/kg 2-DG and 20 mg/kg Ketamine; and (6) 300 mg/kg 2-DG and 20 mg/kg Ketamine (see Fig. 1). One hour prior to FSS, all rats were pre-treated with their respective dose of ketamine, and 30 min later they were injected with their respective dose of 2-DG. Thirty minutes following injections of 2-DG, all rats were exposed to FSS for 10 min. This procedure was carried out for 5 daily sessions of FSS exposure, with each session separated by 24 h (FSS_{1-5}; Fig. 1). Blood was sampled 20 min after FSS_1 (Blood Test 1) and FSS_5 (Blood Test 2) as well as before and 20 min after a drug-free Test Swim (Baseline and Blood Test 3,
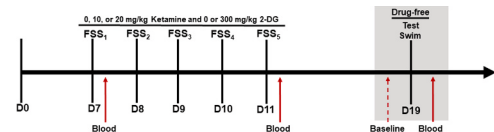

Fig. 1. Experimental design.

respectively) that was performed 7 days after the last consecutive swim session (FSS$_5$). This period was selected because it has been reported that high intensity stressors can alter reactivity to FSS when animals are tested 7 days after stress termination (Belda et al., 2016). The 20 min time point was selected because blood CORT levels peak around this time following FSS (Dal-Zotto et al., 2000).

To control for possible effects of forced swimming stress alone on serum CORT and TNF-α concentrations, blood was sampled at the same timepoints in one additional group of rats in a No Swim stress control condition. These rats were injected with either 0.9% saline only (wt/vol; $n = 6$), or with 300 mg/kg 2-DG only ($n = 6$), or with 20 mg/kg ketamine only ($n = 6$), and were then exposed to dry swim cylinders for FSS$_{1-5}$ and the drug-free Test Swim.

Statistical analysis

Two-way independent or mixed sample design analyses of variance (ANOVA) were used where appropriate. Significant interactions were followed by multiple comparisons using the Bonferroni method. Blood analytes (i.e., CORT and TNF-α) were analyzed using separate two-factor independent design ANOVAs for Blood Test 1 and 2, respectively, while a two-factor mixed design ANOVA was used to compare blood analytes at Baseline and Blood Test 3 in Swim Stress rats. Finally, in No swim stress controls, analytes from all blood samples were compared using a two-factor mixed design ANOVA. One- and two-factor ANOVAs were conducted using SigmaPlot (v.12.5 for Windows) and the threshold of significance was set at $\alpha \leq 0.05$. The exact values of non-significant analyses are not reported.

Results

Corticosterone

Swim stress condition: Fig. 2A represents mean (SEM) serum CORT concentrations on Blood Test 1 in rats pre-treated with 0, 10, or 20 mg/kg ketamine, and 30 min later injected with 0 or 300 mg/kg 2-DG prior to the first session of forced swimming stress (FSS$_1$). The ANOVA revealed a significant main effect of 2-DG dose group [$F(1,47) = 7.302$, $p = 0.01$] and multiple comparisons on marginal means indicated that CORT levels were increased by 2-DG regardless of the ketamine pre-treatment ($p < 0.01$). Fig. 2B represents mean (SEM) serum CORT concentrations on Blood Test 2, and no significant group differences were observed. Fig. 2C represents mean (SEM) serum CORT concentrations at Baseline and on Blood Test 3 following the drug-free Test Swim. The ANOVA revealed a statistically significant interaction between Group and Blood Test [$F(5,95) = 3.228$, $p = 0.015$] as well as a significant main effect of Blood Test [$F(1,95) = 302.731$, $p < 0.001$]. Multiple comparisons further indicated that CORT levels were significantly higher in rats injected with 2-DG in comparison to their vehicle counterparts in the 0 mg/kg ketamine group only ($p < 0.001$), although CORT was significantly elevated on Blood Test 3 in all groups relative to their respective baseline concentrations ($p < 0.001$).

No swim stress condition: Mean (SEM) CORT concentrations observed in rats injected with saline, 20 mg/kg ketamine, or 300 mg/kg 2-DG and not exposed to swim stress are included in Table 1. The ANOVA revealed a statistically significant interaction between Group and Blood Test [$F(6,71) = 3.949$, $p = 0.003$] as well as main effects of Group [$F(2,71) = 4.485$, $p = 0.030$] and Blood Test [$F(3,71) = 3.279$, $p = 0.029$]. Multiple comparisons further indicated that CORT increased on Blood Test 2 in rats administered 5 daily injections of 2-DG compared to rats injected with saline ($p < 0.05$) or ketamine ($p < 0.001$). Moreover, in rats injected with 2-DG, CORT levels were significantly increased on Blood Test 1 in comparison to Baseline ($p < 0.05$), and on Blood Test 2 in comparison to Baseline ($p < 0.001$) and Blood Test 3 ($p < 0.01$). That is, CORT levels significantly increased when rats were administered one or five injections of 2-DG, but when the same rats were
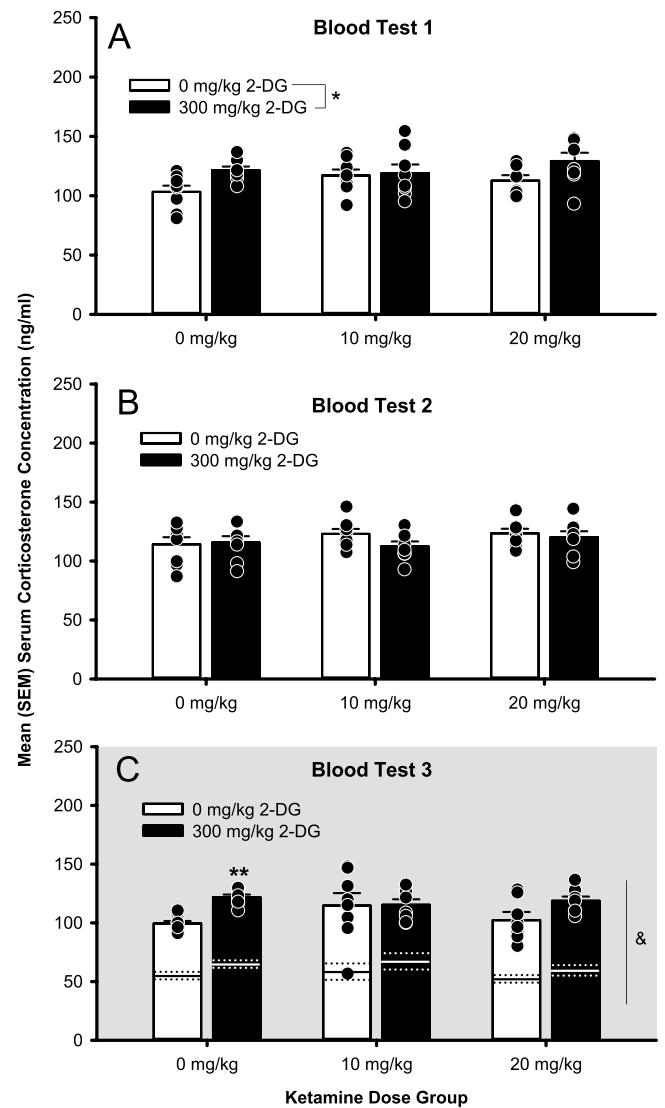

Fig. 2. Mean (SEM) serum corticosterone concentration (ng/ml) on Blood Test 1 (Panel A), Blood Test 2 (Panel B), and Blood Test 3 (Panel C). The solid lines represent mean (SEM = dotted lines) Baseline level of corticosterone within each group. Asterisk (*) indicates a significant main effect of 300 mg/kg 2-DG. Ampersand (&) indicates a significant main effect of swim stress (between Baseline and Blood Test 3; see Fig. 1). Double asterisk (**) indicates a significant difference in comparison to 0 mg/kg 2-DG within ketamine dose group.

tested in a drug-free state 1 week later, CORT concentrations had returned to control levels.

TNF-α

Swim stress condition: Fig. 3A represents mean (SEM) serum TNF-α concentrations on Blood Test 1, and no significant group differences were observed. Fig. 3B represents mean (SEM) serum TNF-α concentrations on Blood Test 2 and again, no significant group differences were observed. Fig. 3C represents mean (SEM) serum TNF-α concentrations at Baseline and on Blood Test 3 following the drug-free Test Swim, and the ANOVA revealed a statistically significant interaction between Group and Blood Test [$F(5,95) = 3.377$, $p = 0.012$] and a significant main effect of Blood Test [$F(1,95) = 19.822$, $p < 0.001$]. Multiple comparisons further indicated that TNF-α levels significantly decreased compared to baseline levels in rats previously administered 5 daily injections of 2-DG on FSS$_{1-5}$ ($p < 0.01$), regardless of their co-administered dose of ketamine. Importantly, rats injected with

Table 1

Mean ± SEM serum CORT and TNF-α concentrations for each Blood Test in rats injected with saline, 20 mg/kg ketamine, or 300 mg/kg 2-DG and exposed to an empty swim tank on FSS$_{1-5}$ as well as the Test Swim (i.e., No swim stress control condition). Values that share a similar bold letter are significantly different from each other. Asterisk indicates a significant difference in the level of TNF-α compared to all other Blood Tests overall.

| Analyte	2-DG dose	Ketamine dose	Blood Test 1	Blood Test 2	Baseline	Blood Test 3*
CORT	0 mg/kg	0 mg/kg	43.43 ± 4.50	49.97 ± 6.83[e]	48.42 ± 10.85	67.07 ± 6.90
	0 mg/kg	20 mg/kg	40.25 ± 9.59	33.96 ± 3.12[d]	32.17 ± 7.05	46.07 ± 5.33
	300 mg/kg	0 mg/kg	64.06 ± 3.91[a]	78.63 ± 10.22[b,c,d,e]	29.95 ± 3.25[a,b]	42.96 ± 12.42[c]
TNF-α	0 mg/kg	0 mg/kg	5.81 ± 0.20	6.01 ± 0.28	6.66 ± 0.71	10.12 ± 1.28
	0 mg/kg	20 mg/kg	6.22 ± 0.23	5.76 ± 0.34	6.64 ± 0.34	10.23 ± 1.01
	300 mg/kg	0 mg/kg	6.81 ± 0.28	6.46 ± 0.41	5.97 ± 0.33	8.73 ± 0.69
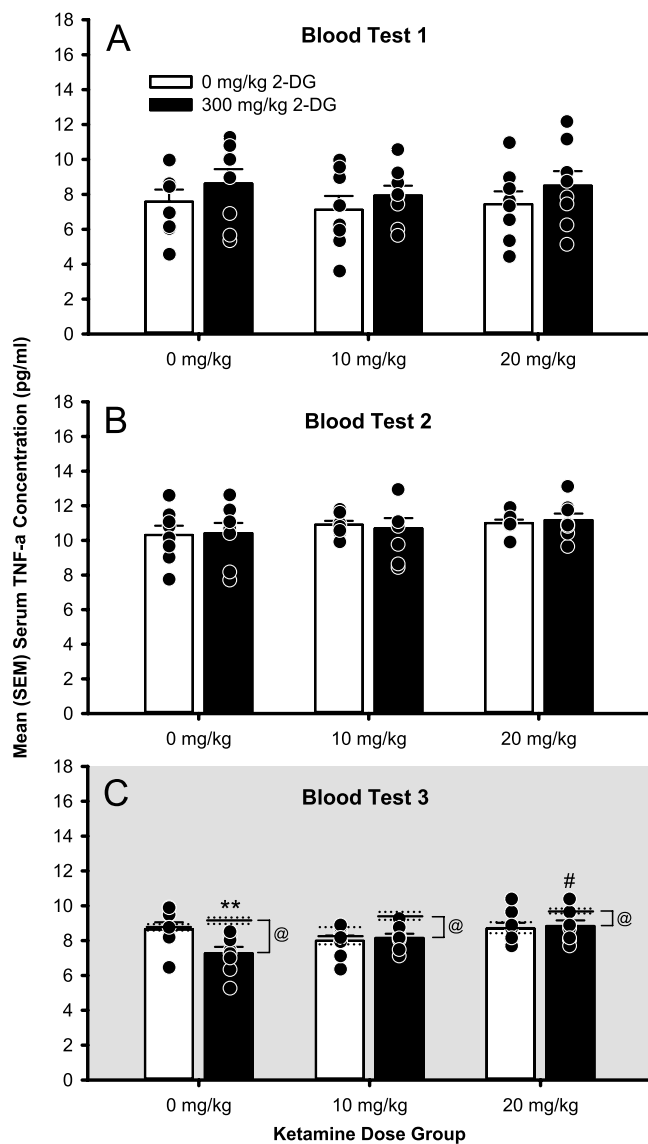
Fig. 3. Mean (SEM) serum TNF-α concentrations (ng/ml) on Blood Test 1 (Panel A), Blood Test 2 (Panel B), and Blood Test 3 (Panel C). The solid lines represent mean (SEM = dotted lines) Baseline level of TNF-α within each group. The at (@) symbol indicates a significant main effect of 300 mg/kg 2-DG dose group. Double asterisk (**) indicates a significant difference in comparison to 0 mg/kg 2-DG within 0 mg/kg ketamine dose group. Pound (#) symbol indicates a significant difference in comparison to rats exposed to 300 mg/kg 2-DG and 0 mg/kg ketamine.

300 mg/kg 2-DG displayed significantly lower levels of TNF-α on Blood Test 3 in comparison to their vehicle counterparts within 0 mg/kg ketamine ($p < 0.05$) and in comparison to rats injected with 300 mg/kg 2-DG and pre-treated with 20 mg/kg ketamine ($p < 0.05$). That is, all rats injected with 2-DG displayed a significant decrease in TNF-α levels from Baseline to Blood Test 3, and the magnitude of this effect was attenuated by pre-treatment with 20 mg/kg ketamine.

No swim stress condition: Mean (SEM) TNF-α concentrations observed in the rats injected with saline, 20 mg/kg ketamine, or 300 mg/kg 2-DG and not exposed to swim stress are reported in Table 1. The ANOVA revealed only a significant main effect of Blood Test [$F(3,71) = 25.760$, $p < 0.001$]. Multiple comparisons on marginal means further indicated that serum TNF-α levels increased overall on Blood Test 3 compared to Blood Test 1, Blood Test 2, and Baseline samples ($p < 0.001$).

Discussion

There is evidence that hypoglycemia can interact with other stressors (Deak et al., 2005; Remus et al., 2015), and that ketamine can mitigate the impact of these stressors on behavior (Melanson et al., 2021) and physiology (Garcia et al., 2009; Tan et al., 2017). The current study in male Sprague-Dawley rats investigated whether pre-treatment with 0, 10, or 20 mg/kg ketamine could modulate the interaction between hypoglycemia induced by 0 or 300 mg/kg 2-deoxy-D-glucose (2-DG) and the psychophysical stress of forced swimming (FSS; 6 sessions, 10 min/session) on serum concentrations of the stress-responsive glucocorticoid CORT (Herman et al., 2016), and the pro-inflammatory cytokine TNF-α (Van Heesch et al., 2013). It was found that the first injection of 2-DG enhanced the CORT response to an initial session of FSS and that this effect dissipated after multiple 2-DG injections/sessions. Moreover, rats displayed significantly higher levels of CORT and lower levels of TNF-α in response to a drug-free Test Swim conducted 1 week after exposure to the combined stressors. Importantly, however, these effects were not observed in rats that received ketamine. Taken together, these findings indicate that ketamine has the potential to reduce the negative impact of interacting stressors on biological reactivity of the HPA axis and immune system.

The current study generated two major findings supporting the idea that ketamine modulated the interaction between hypoglycemia and psychophysical stress. First, the amplified CORT response that was observed in 2-DG-injected rats following exposure to the Test Swim did not occur in animals that were treated with ketamine (Fig. 2, Panel C). This finding is consistent with the interpretation that ketamine exhibits anti-stress effects (Amat et al., 2016), is prophylactic against future stressors (Brachman et al., 2016; Mastrodonato et al., 2020; Parise et al., 2013), and can normalize output of the HPA axis up to 1 week following repeated stress (Garcia et al., 2009). Second, TNF-α levels decreased following the Test Swim in all rats exposed to multiple injections of 2-DG, and the magnitude of this suppression was significantly reduced by ketamine (Fig. 3, Panel C). This finding is consistent with the suggestion that ketamine can regulate the immune response to various stressors (Chang et al., 2016; Mastrodonato et al., 2020). But, while previous studies have emphasized the ability of ketamine to reduce inflammation caused by various forms of stress (Chang et al., 2010; Clarke et al., 2017; Zhang et al., 2016), the current findings provide first-time evidence that prophylactic ketamine can also block

stress-induced immunosuppression (Connor et al., 2005; Hou et al., 2016).

While the exact mechanisms underlying ketamine's modulation of these responses remain unclear, there are several possibilities. First, it has been found that NMDA receptor antagonists can block hypoglycemia-induced lesions to stress-sensitive brain regions (Tasker et al., 1992; Wieloch, 1985) and that ketamine, also an NMDA receptor antagonist, can reverse deficits in glucocorticoid receptor (GR) density caused by repeated stress (Fraga et al., 2021). Because lesions (Herman et al., 2016) or knockdown of GRs (McKlveen et al., 2013) in stress-sensitive regions of the brain amplify the CORT response to acute stress, it is possible that ketamine may have preserved HPA output in the current study by maintaining cellular and/or molecular mechanisms of negative feedback of the HPA axis (Herman et al., 2016). Second, ketamine may have directly modulated lymphocyte activity as NMDA receptor density is increased on lymphocytes activated by hypoglycemia (Iqbal et al., 2019) and is associated with suppression of pro-inflammatory cytokine release in vitro (Mashkina et al., 2010). Third, ketamine can reduce serum levels of soluble TNF-α receptor-1 (sTNFR1; Park et al., 2017), which is known to sequester TNF-α and cause reductions of this cytokine in the blood (Engelmann et al., 1990; Idriss and Naismith, 2000). Moreover, there is evidence that individuals with impaired glucose metabolism display elevated plasma levels of sTNFR1 (Mattisson et al., 2017), suggesting that prolonged activity of sTNFR1 and/or lymphocytes may have played a role in the suppressed TNF-α response induced by 2-DG and the effects of ketamine observed in this study.

Previous studies have reported that swim stress alone (Dal-Zotto et al., 2000; Himmerich et al., 2013) and 2-DG alone (Deak et al., 2005; Dréau et al., 2000; Horman et al., 2018) can increase blood levels of CORT and TNF-α in rodents. In the current study, serum levels of CORT increased following exposure to swim stress and to injections of 2-DG (Table 1), which is consistent with previous studies reporting increased CORT levels in response to forced swimming (Dal-Zotto et al., 2000) and experimentally-induced hypoglycemia (Deak et al., 2005; Horman et al., 2018; Razavi Nematollahi et al., 2009). Neither stressor alone significantly impacted TNF-α levels. When these stressors were combined, the CORT response was significantly enhanced as early as the first session of FSS (Blood Test 1; Fig. 2, Panel A) and, when the same animals were tested 1 week later, those exposed to the combined stressors displayed an amplified CORT response (Fig. 2, Panel C) and a suppressed TNF-α response (Fig. 3, Panel C). These findings are consistent with the idea that different stressor types can interact to dysregulate the CORT and inflammatory responses (Deak et al., 2005; Lovelock and Deak, 2017), and that exposure to high-intensity stressors can amplify the CORT response to brief, psychophysical stress (Belda et al., 2016). Interestingly, the differences in physiological response observed on the Test Swim in comparison to Blood Test 1 and 2 may have been the result of time-dependent sensitization (Antelman et al., 2000), a form of metaplastic adaptation that generates an amplified physiological response with the passage of time between an initiating (i.e., interacting 2-DG and FSS) and challenging (i.e., Test Swim) stressor (Bell and Koithan, 2012). Thus, it is possible that time-dependent sensitization of biological processes involved in HPA axis activation (Harvey et al., 2003; Sorg et al., 1994) and stress-induced immunomodulation (Frank et al., 2007, 2012; Golovatscka et al., 2012; Miller et al., 2019) contributed to the amplified CORT and TNF-α responses in the current study. Nevertheless, these novel findings provide potential physiological mechanisms that may underlmy the combined effect of hypoglycemic and psychophysical stressors on depressive-like behaviors previously reported by Melanson et al. (2021).

It should be noted that although CORT levels were unaffected by ketamine on Blood Tests 1 and 2 (Fig. 2, Panels B & C), it has been reported that ketamine can both reduce CORT (Juven-Wetzler et al., 2014) and elevate CORT (Radford et al., 2020) in rats. These apparently discrepant findings are likely to result from differences in the timing of blood collection and duration of administration. Specifically, Juven-Wetzler et al. (2014) examined CORT levels at 20 min following an IP injection of ketamine, while Radford et al. (2020) assessed CORT immediately after cessation of a 2 h ketamine infusion. Importantly, this increase in CORT following a ketamine infusion was not evident when CORT levels were re-assessed at 2 h post-administration (Radford et al., 2018), which is similar to the findings in the current study as blood samples were collected at least 80 min following the injection of ketamine. Moreover, in the current study, ketamine alone had no effect on CORT levels in the No Swim stress condition (Table 1), which suggests that if ketamine does impact CORT levels as previously described, this effect may be time-dependent and limited to the first hour following drug administration.

Overall, the current study demonstrates, for the first time, that ketamine can prevent the impact of interacting hypoglycemic and psychophysical stressors on CORT and TNF-α responses, further supporting its application in the treatment of psychiatric conditions characterized by HPA axis hyperreactivity and altered immune profiles, such as depression (Kiraly et al., 2017; Swaab et al., 2005). As well, the current data also point towards a possible mechanistic link in comorbid metabolic and mood disorders, like diabetes and depression (Pashaki et al., 2019). Finally, the current data, in combination with behavioral data reported by Melanson et al. (2021), provide a potential physiological basis by which ketamine could exhibit its antidepressant and prophylactic effects, and further suggests CORT and TNF-α as potential biological markers of ketamine's treatment response in depressed individuals (Rong et al., 2018).

CRediT authorship contribution statement

Brett Melanson: Investigation, Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Visualization, Project administration. **Francesco Leri**: Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Conflict of Interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.Ethical statement:All experiments were carried out in accordance with the guidelines of the Canadian Council on Animal Care and approved by the Animal Care Committee of the University of Guelph (Ontario, Canada).

References

Amat, J., Dolzani, S.D., Tilden, S., Christianson, J.P., Kubala, K.H., Bartholomay, K., Sperr, K., Ciancio, N., Watkins, L.R., Maier, S.F., 2016. Previous ketamine produces an enduring blockade of neurochemical and behavioral effects of uncontrollable stress. J. Neurosci. 36 (1), 153–161. https://doi.org/10.1523/JNEUROSCI.3114-15.2016.

Anderson, R.J., Freedland, K.E., Clouse, R.E., Lustman, P.J., 2001. The prevalence of comorbid depression in adults with diabetes: a meta-analysis. Diabetes Care 24, 1069–1078. https://doi.org/10.2337/diacare.24.6.1069.

Antelman, S.M., Levine, J., Gershon, S., 2000. Time-dependent sensitization: the odyssey of a scientific heresy from the laboratory to the door of the clinic. Mol. Psychiatry 5 (4), 350–356. https://doi.org/10.1038/sj.mp.4000721.

Balkan, B., Gozen, O., Koylu, E.O., Keser, A., Kuhar, M.J., Pogun, S., 2012. Region- and sex-specific changes in CART mRNA in rat hypothalamic nuclei induced by forced swim stress. Brain Res. 1479, 62–71. https://doi.org/10.1016/j.brainres.2012.08.043.

Belda, X., Nadal, R., Armario, A., 2016. Critical features of acute stress-induced cross-sensitization identified through the hypothalamic-pituitary-adrenal axis output. Sci. Rep. 6 (March), 1–12. https://doi.org/10.1038/srep31244.

Bell, I.R., Koithan, M., 2012. A model for homeopathic remedy effects: low dose nanoparticles, allostatic cross-adaptation, and time-dependent sensitization in a complex adaptive system. BMC Complement. Altern. Med. 12, 191. https://doi.org/10.1186/1472-6882-12-191.

Borsoi, M., Antonio, C.B., Müller, L.G., Viana, A.F., Hertzfeldt, V., Lunardi, P.S., Zanotto, C., Nardin, P., Ravazzolo, A.P., Rates, S.M.K., Gonçalves, C.A., 2015. Repeated forced swimming impairs prepulse inhibition and alters brain-derived neurotrophic factor and astroglial parameters in rats. Pharmacol. Biochem. Behav. 128, 50–61. https://doi.org/10.1016/j.pbb.2014.11.012.

Brachman, R.A., McGowan, J.C., Perusini, J.N., Lim, S.C., Pham, T.H., Faye, C., Gardier, A.M., Mendez-David, I., David, D.J., Hen, R., Denny, C.A., 2016. Ketamine as a prophylactic against stress-induced depressive-like behavior. Biol. Psychiatry 79 (9), 776–786. https://doi.org/10.1016/j.biopsych.2015.04.022.

Carrier, N., Kabbaj, M., 2013. Sex differences in the antidepressant-like effects of ketamine. Neuropharmacology 70, 27–34. https://doi.org/10.1016/j.neuropharm.2012.12.009.

Carroll, J.E., Low, C.A., Prather, A.A., Cohen, S., Fury, J.M., Ross, D.C., Marsland, A.L., 2011. Negative affective responses to a speech task predict changes in interleukin (IL)-6. Brain Behav. Immun. 25 (2), 232–238. https://doi.org/10.1016/j.bbi.2010.09.024.

Chang, E.I., Zárate, M.A., Rabaglino, M.B., Richards, E.M., Keller-Wood, M., Wood, C.E., 2016. Ketamine suppresses hypoxia-induced inflammatory responses in the late-gestation ovine fetal kidney cortex. J. Physiol. 594 (5), 1295–1310. https://doi.org/10.1113/JP271066.

Chang, H.C., Lin, K.H., Tai, Y.T., Chen, J.T., Chen, R.M., 2010. Lipoteichoic acid-induced tnf-α and il-6 gene expressions and oxidative stress production in macrophages are suppressed by ketamine through downregulating toll-like receptor 2-mediated activation of erk1/2 and nfκb. Shock 33 (5), 485–492. https://doi.org/10.1097/SHK.0b013e3181c3cea5.

Clarke, M., Razmjou, S., Prowse, N., Dwyer, Z., Litteljohn, D., Pentz, R., Anisman, H., Hayley, S., 2017. Ketamine modulates hippocampal neurogenesis and pro-inflammatory cytokines but not stressor induced neurochemical changes. Neuropharmacology 112, 210–220. https://doi.org/10.1016/j.neuropharm.2016.04.021.

Connor, T.J., Brewer, C., Kelly, J.P., Harkin, A., 2005. Acute stress suppresses pro-inflammatory cytokines TNF-α and IL-1β independent of a catecholamine-driven increase in IL-10 production. J. Neuroimmunol. 159 (1–2), 119–128. https://doi.org/10.1016/j.jneuroim.2004.10.016.

Dal-Zotto, S., Martí, O., Armario, A., 2000. Influence of single or repeated experience of rats with forced swimming on behavioural and physiological responses to the stressor. Behav. Brain Res. 114 (1–2), 175–181. https://doi.org/10.1016/S0166-4328(00)00220-5.

De Alvaro, C., Teruel, T., Hernandez, R., Lorenzo, M., 2004. Tumor necrosis factor α produces insulin resistance in skeletal muscle by activation of inhibitor κB kinase in a p38 MAPK-dependent manner. J. Biol. Chem. 279 (17), 17070–17078. https://doi.org/10.1074/jbc.M312021200.

Deak, T., Bordner, K.A., McElderry, N.K., Barnum, C.J., Blandino, P., Deak, M.M., Tammariello, S.P., 2005. Stress-induced increases in hypothalamic IL-1: a systematic analysis of multiple stressor paradigms. Brain Res. Bull. 64 (6), 541–556. https://doi.org/10.1016/j.brainresbull.2004.11.003.

Dréau, D., Morton, D.S., Foster, M., Fowler, N., Sonnenfeld, G., 2000. Effects of 2-deoxy-D-glucose administration on cytokine production in BDF1 mice. J. Interferon Cytokine Res. Off. J. Int. Soc. Interferon Cytokine Res. 20, 247–255. https://doi.org/10.1089/107999000312667.

Drummond, J.B., Barbosa, I.G., Dantzer, R., Teixeira, A.L., 2018. The effect of insulin-induced hypoglycemia on inflammatory markers: a systematic review. Brain Behav. Immun. 73 (January), 41–50. https://doi.org/10.1016/j.bbi.2018.05.003.

Dunn, A., 2006. Cytokine activation of the HPA axis. Ann. N. Y. Acad. Sci. 917 (1), 608–617. https://doi.org/10.1111/j.1749-6632.2000.tb05426.x.

Engelmann, H., Novick, D., Wallachs, D., 1990. Two tumor necrosis factor-binding proteins purified from human urine. Evidence for immunological cross-reactivity with cell surface tumor necrosis factor receptors. J. Biol. Chem. 265 (3) https://doi.org/10.1016/S0021-9258(19)40049-5.

Engelsen, B., Westerberg, E., Fonnum, F., Wieloch, T., 1986. Effect of insulin-induced hypoglycemia on the concentrations of glutamate and related amino acids and energy metabolites in the intact and decorticated rat neostriatum. J. Neurochem. 47 (5), 1634–1641. https://doi.org/10.1111/j.1471-4159.1986.tb00806.x.

Fraga, D.B., Camargo, A., Olescowicz, G., Padilha, D.A., Mina, F., Budni, J., Brocardo, P. S., Rodrigues, A.L.S., 2021. Ketamine, but not fluoxetine, rapidly rescues corticosterone-induced impairments on glucocorticoid receptor and dendritic branching in the hippocampus of mice. Metab. Brain Dis. https://doi.org/10.1007/s11011-021-00743-2.

Frank, M.G., Baratta, M.V., Sprunger, D.B., Watkins, L.R., Maier, S.F., 2007. Microglia serve as a neuroimmune substrate for stress-induced potentiation of CNS pro-inflammatory cytokine responses. Brain Behav. Immun. 21, 47–59. https://doi.org/10.1016/j.bbi.2006.03.005.

Frank, M.G., Thompson, B.M., Watkins, L.R., Maier, S.F., 2012. Glucocorticoids mediate stress-induced priming of microglial pro-inflammatory responses. Brain Behav. Immun. 26 (2), 337–345. https://doi.org/10.1016/j.bbi.2011.10.005.

Gao, M., Rejaei, D., Liu, H., 2016. Ketamine use in current clinical practice. Acta Pharmacol. Sin. 37 (7), 865–872. https://doi.org/10.1038/aps.2016.5.

Garcia, L.S.B., Comim, C.M., Valvassori, S.S., Réus, G.Z., Stertz, L., Kapczinski, F., Gavioli, E.C., Quevedo, J., 2009. Ketamine treatment reverses behavioral and physiological alterations induced by chronic mild stress in rats. Prog. Neuro-psychopharmacol. Biol. Psychiatry 33 (3), 450–455. https://doi.org/10.1016/j.pnpbp.2009.01.004.

Gold, A.E., MacLeod, K.M., Frier, B.M., Deary, I.J., 1995. Changes in mood during acute hypoglycemia in healthy participants. J. Personal. Soc. Psychol. 68, 498–504. https://doi.org/10.1037/0022-3514.68.3.498.

Golovatscka, V., Ennes, H., Mayer, E.A., Bradesi, S., 2012. Chronic stress-induced changes in pro-inflammatory cytokines and spinal glia markers in the rat: a time course study. NeuroImmunoModulation 19, 367–376. https://doi.org/10.1159/000342092.

Gonçalves, C.L., Abelaira, H.M., Rosa, T., de Moura, A.B., Veron, D.C., Borba, L.A., Botelho, M.E.M., Goldim, M.P., Garbossa, L., Fileti, M.E., Petronilho, F., Ignácio, Z. M., Quevedo, J., Réus, G.Z., 2021. Ketamine treatment protects against oxidative damage and the immunological response induced by electroconvulsive therapy. Pharmacol. Rep. 73 (2), 525–535. https://doi.org/10.1007/s43440-020-00200-4.

Harrell, C.S., Gillespie, C.F., Neigh, G.N., 2016. Energetic stress: the reciprocal relationship between energy availability and the stress response. Physiol. Behav. 166, 43–55. https://doi.org/10.1016/j.physbeh.2015.10.009.

Harvey, B.H., Naciti, C., Brand, L., Stein, D.J., 2003. Changes evoked by a time-dependent sensitisation (TDS) stress model in rats. Brain Res. 983, 97–107. ⟨www.elsevier.com⟩.

Herman, J.P., Mcklveen, J.M., Ghosal, S., Kopp, B., Wulsin, A., Makinson, R., Scheimann, J., Myers, B., 2016. Regulation of the hypothalamic-pituitary-adrenocortical stress response. Comprehensive Physiology. Compr. Physiol. 6 (2), 603–621. https://doi.org/10.1002/cphy.c150015.Regulation.

Himmerich, H., Fischer, J., Bauer, K., Kirkby, K.C., Sack, U., Krügel, U., 2013. Stress-induced cytokine changes in rats. Eur. Cytokine Netw. 24 (2), 97–103. https://doi.org/10.1684/ecn.2013.0338.

Horman, T., Fernandes, M.F., Zhou, Y., Fuller, B., Tigert, M., Leri, F., 2018. An exploration of the aversive properties of 2-deoxy-D-glucose in rats. Psychopharmacology 235, 3055–3063. https://doi.org/10.1007/s00213-018-4998-1.

Hou, D.D., Zhu, R.Z., Sun, Z., Ma, X.D., Wang, D.C., Timothy, H., Chen, W.N., Yan, F., Lei, P., Han, X.W., Chen, D.X., Cai, L.P., Guan, H.Q., 2016. Serum proteomics analysis in rats of immunosuppression induced by chronic stress. Scand. J. Immunol. 84 (3), 165–173. https://doi.org/10.1111/sji.12461.

Idriss, H.T., Naismith, J.H., 2000. TNFα and the TNF receptor superfamily: structure-function relationship(s). Microsc. Res. Tech. 50, 184–195.

Imbe, H., Kimura, A., 2015. Repeated forced swim stress prior to complete Freund's adjuvant injection enhances mechanical hyperalgesia and attenuates the expression of pCREB and δFosB and the acetylation of histone H3 in the insular cortex of rat. Neuroscience 301, 12–25. https://doi.org/10.1016/j.neuroscience.2015.05.065.

Iqbal, A., Prince, L.R., Novodvorsky, P., Bernjak, A., Thomas, M.R., Birch, L., Lambert, D., Kay, L.J., Wright, F.J., Macdonald, I.A., Jacques, R.M., Storey, R.F., Mccrimmon, R. J., Francis, S., Heller, S.R., Sabroe, I., 2019. Effect of hypoglycemia on inflammatory responses and the response to low-dose endotoxemia in humans. J. Clin. Endocrinol. Metab. 104 (4), 1187–1199. https://doi.org/10.1210/jc.2018-01168.

Juven-Wetzler, A., Cohen, H., Kaplan, Z., Kohen, A., Porat, O., Zohar, J., 2014. Immediate ketamine treatment does not prevent posttraumatic stress responses in an animal model for PTSD. Eur. Neuropsychopharmacol. 24 (3), 469–479. https://doi.org/10.1016/j.euroneuro.2013.08.007.

Kiraly, D.D., Horn, S.R., Van Dam, N.T., Costi, S., Schwartz, J., Kim-Schulze, S., Patel, M., Hodes, G.E., Russo, S.J., Merad, M., Iosifescu, D.V., Charney, D.S., Murrough, J.W., 2017. Altered peripheral immune profiles in treatment-resistant depression: response to ketamine and prediction of treatment outcome. Transl. Psychiatry 7 (3), e1065. https://doi.org/10.1038/tp.2017.31.

Kuo, T., McQueen, A., Chen, T.C., Wang, J.C., 2015. Regulation of glucose homeostasis by glucocorticoids. Adv. Exp. Med. Biol. 872, 99–126. https://doi.org/10.1007/978-1-4939-2895-8_5.

López-López, A.L., Bonilla, H.J., Escobar Villanueva, M., del, C., Brianza, M.P., Vázquez, G.P., Alarcón, F.J.A., 2016. Chronic unpredictable mild stress generates oxidative stress and systemic inflammation in rats. Physiol. Behav. 161, 15–23. https://doi.org/10.1016/j.physbeh.2016.03.017.

Lorenzo, M., Fernández-Veledo, S., Vila-Bedmar, R., Garcia-Guerra, L., De Alvaro, C., Nieto-Vazquez, I., 2008. Insulin resistance induced by tumor necrosis factor-alpha in myocytes and brown adipocytes. J. Anim. Sci. 86 (14 Suppl), E94–E104.

Lovelock, D.F., Deak, T., 2017. Repeated exposure to two stressors in sequence demonstrates that corticosterone and paraventricular nucleus of the hypothalamus interleukin-1β responses habituate independently. J. Neuroendocrinol. 29 https://doi.org/10.1111/jne.12514.

Lustman, P.J., Clouse, R.E., 2005. Depression in diabetic patients: the relationship between mood and glycemic control. J. Diabetes Complicat. 19 (2), 113–122. https://doi.org/10.1016/j.jdiacomp.2004.01.002.

Mashkina, A.P., Cizkova, D., Vanicky, I., Boldyrev, A.A., 2010. NMDA receptors are expressed in lymphocytes activated both in vitro and in vivo. Cell. Mol. Neurobiol. 30 (6), 901–907. https://doi.org/10.1007/s10571-010-9519-7.

Mastrodonato, A., Cohensedgh, O., LaGamma, C.T., McGowan, J.C., Hunsberger, H.C., Denny, C.A., 2020. Prophylactic (R,S)-ketamine selectively protects against

inflammatory stressors. Behav. Brain Res. 378 (July 2019), 112238 https://doi.org/10.1016/j.bbr.2019.112238.

Mattisson, I.Y., Björkbacka, H., Wigren, M., Edsfeldt, A., Melander, O., Fredrikson, G.N., Bengtsson, E., Gonçalves, I., Orho-Melander, M., Engström, G., Almgren, P., Nilsson, J., 2017. Elevated markers of death receptor-activated apoptosis are associated with increased risk for development of diabetes and cardiovascular disease. EBioMedicine 26, 187–197. https://doi.org/10.1016/j.ebiom.2017.11.023.

McKlveen, J.M., Myers, B., Flak, J.N., Bundzikova, J., Solomon, M.B., Seroogy, K.B., Herman, J.P., 2013. Role of prefrontal cortex glucocorticoid receptors in stress and emotion. Biol. Psychiatry 74 (9), 672–679. https://doi.org/10.1016/j.biopsych.2013.03.024.

Melanson, B., Lapointe, T., Leri, F., 2021. Impact of impaired glucose metabolism on responses to a psychophysical stressor: modulation by ketamine. Psychopharmacology 238 (4), 1005–1015. https://doi.org/10.1007/s00213-020-05748-w.

Miller, E.S., Apple, C.G., Kannan, K.B., Funk, Z.M., Plazas, J.M., Efron, P.A., Mohr, A.M., 2019. Chronic stress induces persistent low-grade inflammation. Am. J. Surg. 218 (4), 677–683. https://doi.org/10.1016/j.amjsurg.2019.07.006.

Myers, B., Mcklveen, J.M., Herman, J.P., 2013. Glucocorticoid actions on synapses, circuits, and behavior: implications for the energetics of stress. https://doi.org/10.1016/j.yfrne.2013.12.003.

Niciu, M.J., Shovestul, B.J., Jaso, B.A., Farmer, C., Luckenbaugh, D.A., Brutsche, N.E., Park, L.T., Ballard, E.D., Zarate, C.A., 2018. Features of dissociation differentially predict antidepressant response to ketamine in treatment-resistant depression. J. Affect. Disord. 232 (January), 310–315. https://doi.org/10.1016/j.jad.2018.02.049.

Nouwen, A., Adriaanse, M.C., van Dam, K., Iversen, M.M., Viechtbauer, W., Peyrot, M., Caramlau, I., Kokoszka, A., Kanc, K., de Groot, M., Nefs, G., Pouwer, F., 2019. Longitudinal associations between depression and diabetes complications: a systematic review and meta-analysis. Diabet. Med. 36 (12), 1562–1572. https://doi.org/10.1111/dme.14054.

Parise, E.M., Alcantara, L.F., Warren, B.L., Wright, K.N., Hadad, R., Sial, O.K., Kroeck, K.G., Iñiguez, S.D., Bolaños-Guzmán, C.A., 2013. Repeated ketamine exposure induces an enduring resilient phenotype in adolescent and adult rats. Biol. Psychiatry 74 (10), 750–759. https://doi.org/10.1016/j.biopsych.2013.04.027.

Park, M., Newman, L.E., Gold, P.W., Luckenbaugh, D.A., Yuan, P., Machado-Vieira, R., Zarate, C.A., 2017. Change in cytokine levels is not associated with rapid antidepressant response to ketamine in treatment-resistant depression. J. Psychiatr. Res. 84, 113–118. https://doi.org/10.1016/j.jpsychires.2016.09.025.

Pashaki, M.S., Mezel, J.A., Mokhtari, Z., Gheshlagh, R.G., Hesabi, P.S., Nematifard, T., Khaki, S., 2019. The prevalence of comorbid depression in patients with diabetes: a meta-analysis of observational studies. Diabetes Metab. Syndr. Clin. Res. Rev. 13 (6), 3113–3119. https://doi.org/10.1016/j.dsx.2019.11.003.

Phillips, J.L., Norris, S., Talbot, J., Birmingham, M., Hatchard, T., Ortiz, A., Owoeye, O., Batten, L.A., Blier, P., 2019. Single, repeated, and maintenance ketamine infusions for treatment-resistant depression: a randomized controlled trial. Am. J. Psychiatry 176 (5), 401–409. https://doi.org/10.1176/appi.ajp.2018.18070834.

Radford, K.D., Park, T.Y., Osborne-Smith, L., Choi, K.H., 2018. Effects of subanesthetic intravenous ketamine infusion on corticosterone and brain-derived neurotrophic factor in the plasma of male Sprague-Dawley rats. AANA J. 86 (5), 393–400.

Radford, K.D., Spencer, H.F., Zhang, M., Berman, R.Y., Girasek, Q.L., Choi, K.H., 2020. Association between intravenous ketamine-induced stress hormone levels and long-term fear memory renewal in Sprague-Dawley rats. Behav. Brain Res. 378 (June 2019), 112259 https://doi.org/10.1016/j.bbr.2019.112259.

Razavi Nematollahi, L., Kitabchi, A.E., Stentz, F.B., Wan, J.Y., Larijani, B.A., Tehrani, M.M., Gozashti, M.H., Omidfar, K., Taheri, E., 2009. Proinflammatory cytokines in response to insulin-induced hypoglycemic stress in healthy subjects. Metab. Clin. Exp. 58 (4), 443–448. https://doi.org/10.1016/j.metabol.2008.10.018.

Remus, J.L., Stewart, L.T., Camp, R.M., Novak, C.M., Johnson, J.D., 2015. Interaction of metabolic stress with chronic mild stress in altering brain cytokines and sucrose preference. Behav. Neurosci. 129 (3), 321–330. https://doi.org/10.1037/bne0000056.

Rong, C., Park, C., Rosenblat, J.D., Subramaniapillai, M., Zuckerman, H., Fus, D., Lee, Y.L., Pan, Z., Brietzke, E., Mansur, R.B., Cha, D.S., Lui, L.M.W., McIntyre, R.S., 2018. Predictors of response to ketamine in treatment resistant major depressive disorder and bipolar disorder. Int. J. Environ. Res. Public Health 15 (4). https://doi.org/10.3390/ijerph15040771.

Sorg, B.A., Hooks, M.S., Kalivas, P.W., 1994. Neuroanatomy and neurochemical mechanisms of time-dependent sensitization. Toxicol. Ind. Health 10 (4), 369–386.

Swaab, D.F., Bao, A.M., Lucassen, P.J., 2005. The stress system in the human brain in depression and neurodegeneration. Ageing Res. Rev. 4, 141–194. https://doi.org/10.1016/j.arr.2005.03.003.

Tan, S., Wang, Y., Chen, K., Long, Z., Zou, J., 2017. Ketamine alleviates depressive-like behaviors via down-regulating inflammatory cytokines induced by chronic restraint stress in mice. Biol. Pharm. Bull. 40 (8), 1260–1267. https://doi.org/10.1248/bpb.b17-00131.

Tasker, R.C., Coyle, J.T., Vornov, J.J., 1992. The regional vulnerability to hypoglycemia-induced neurotoxicity in organotypic hippocampal culture: protection by early tetrodotoxin or delayed MK-801. J. Neurosci. 12 (11), 4298–4308. https://doi.org/10.1523/jneurosci.12-11-04298.1992.

Timberlake II, M., Roy, B., Dwivedi, Y., 2019. A novel animal model for studying depression featuring the induction of the unfolded protein response in hippocampus. Mol. Neurobiol. 56, 8524–8536. https://doi.org/10.1007/s12035-019-01687-6.

Van Heesch, F., Prins, J., Korte-Bouws, G.A.H., Westphal, K.G.C., Lemstra, S., Olivier, B., Kraneveld, A.D., Korte, S.M., 2013. Systemic tumor necrosis factor-alpha decreases brain stimulation reward and increases metabolites of serotonin and dopamine in the nucleus accumbens of mice. Behav. Brain Res. 253, 191–195. https://doi.org/10.1016/j.bbr.2013.07.038.

Walker, A.J., Foley, B.M., Sutor, S.L., McGillivray, J.A., Frye, M.A., Tye, S.J., 2015. Peripheral proinflammatory markers associated with ketamine response in a preclinical model of antidepressant-resistance. Behav. Brain Res. 293, 198–202. https://doi.org/10.1016/j.bbr.2015.07.026.

Wieloch, T., 1985. Hypoglycemia-induced neuronal damage prevented by an N-methyl-D-aspartate antagonist. Science 230 (4726), 681–683. https://doi.org/10.1126/science.2996146.

World Health Organization, 2016. Global Report on Diabetes. Isbn. https://doi.org/ISBN 978 92 4 156525 7.

World Health Organization, 2017. Depression and Other Common Mental Disorders: Global Health Estimates. World Health Organization, pp. 1–24.

Zhan, Y., Zhou, Y., Zheng, W., Liu, W., Wang, C., Lan, X., Deng, X., Xu, Y., Zhang, B., Ning, Y., 2020. Alterations of multiple peripheral inflammatory cytokine levels after repeated ketamine infusions in major depressive disorder. Transl. Psychiatry 10, 246. https://doi.org/10.1038/s41398-020-00933-z.

Zhang, G.F., Wang, J., Han, J.F., Guo, J., Xie, Z.M., Pan, W., Yang, J.J., Sun, K.J., 2016. Acute single dose of ketamine relieves mechanical allodynia and consequent depression-like behaviors in a rat model. Neurosci. Lett. 631, 7–12. https://doi.org/10.1016/j.neulet.2016.08.006.